

# ✓ OPTIMIZATION OF A BIOCHEMICAL FED-BATCH REACTOR USING PULSE FEEDS

*A Thesis Submitted  
in Partial Fulfillment of the Requirements  
for the Degree of  
MASTER OF TECHNOLOGY*

by  
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to the  
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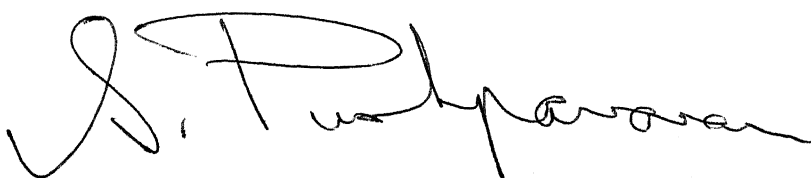


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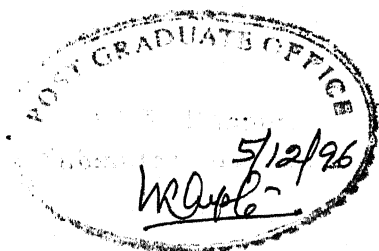
Certified that the work contained in the thesis entitled "Optimization of a Biochemical Fed-Batch Reactor Using Pulse Feeds", by V.C.L.N. Murthy, has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.



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V.C.L.N. Murthy

# CONTENTS

	page
Certificate	ii
Acknowledgement	iii
Contents	iv
List of Tables	v
List of Figures	vi
Nomenclature	vii
Abstract	ix
1. Introduction	1
2. Problem Formulation and Method of Solution	4
2.1 Methods of solution	5
2.2 Sequential Quadratic Programming	10
3.Results and Discussion	15
3.1 Part - 1	15
3.2 Part - 2	21
4. Conclusions	25
REFERENCES	26

# LIST OF TABLES

Table	Title	Page
1	Results obtained using the integral method	28
2	Different cases depending on the ordering of $S_0$ , $S_F$ , $S^*$	29
3	Results of fixed $t_f$ and $V_f$ , with equal sub-intervals	30
4	Results of fixed $t_f$ and fixed $V_f$ , with unequal sub-intervals	31
5	Results of fixed $t_f$ and free $V_f$ , with equal sub-intervals	32
6	Results of fixed $t_f$ and free $V_f$ , with unequal sub-intervals	33
7	Results of free $t_f$ and fixed $V_f$ , with unequal sub-intervals	34
8	Results of free $t_f$ and free $V_f$ , with unequal sub-intervals	35
9	Results of free $t_f$ and fixed $V_f$ , with equal sub-intervals	36
10	Results of free $t_f$ and free $V_f$ , with equal sub-intervals	37

# LIST OF FIGURES

Figure	Title	Page
1	The variables in the different stages of a cycle	14
2a	Volume profile for integral method $N = 5$	38
2b	Bio mass concentration profile for integral method $N = 5$	39
3a	Volume profile for the fixed $V_f$ and $t_f$ with equal intervals	40
3b(i)	Bio mass concentration profile for the fixed $V_f$ and $t_f$ with equal intervals	41
3b(ii)	Substrate concentration profile for the fixed $V_f$ and $t_f$ with equal intervals	42
4a	Volume profile for the free $V_f$ and $t_f$ with unequal intervals	43
4b(i)	Bio mass concentration profile for the free $V_f$ and $t_f$ with unequal intervals	44
4b(ii)	Substrate concentration profile for the free $V_f$ and $t_f$ with unequal intervals	45

## Nomenclature

$F$	:	Volumetric flow-rate (L/hr)
$K_m, K_i$	:	Haldane kinetic parameters
$N$	:	No. of stages
$r$	:	Ratio of two successive volumes of the reactor
$S$	:	Substrate concentration (g/L)
$S_F$	:	Feed substrate concentration (g/L)
$S_O$	:	Initial substrate concentration (g/L)
$S^*$	:	The substrate concentration at which the reaction rate is a maximum
$t$	:	Time (hr)
$t_f$	:	Final time (hr)
$\Delta t$	:	Duration of sub-interval (hr)
$u$	:	Biomass concentration immediately after the addition of each pulse
$V$	:	Volume of the reactor (L)
$V_O$	:	Initial volume of the reactor (L)
$V_f$	:	Final volume of the reactor (L)
$\Delta V$	:	Volume of pulse (L)
$X$	:	Biomass concentration (g/L)
$X_O$	:	Initial biomass concentration (g/L)
$X_f$	:	Final biomass concentration (g/L)
$Y$	:	Constant yield

### Greek:

$\mu$	:	Specific growth rate ( $\text{hr}^{-1}$ )
$\mu_m$	:	Specific growth rate constant



### Subscripts:

o : Initial  
f : Final  
F : Feed  
i :  $i^{\text{th}}$  stage

### Superscripts:

- : At the end of each sub-interval  
+ : After the addition of the pulse, or at the beginning of a sub interval.  
\* : maximum

## Abstract

In this work we analyse the optimal control problem of a biochemical reactor sustaining a reaction characterised by substrate inhibition. The control variable is the substrate feed rate. We approximate the feed rate policy by discrete pulses. The entire period of operation is divided into sub-intervals. The substrate is assumed to be added at the ~~end~~<sup>beginning</sup> of each sub-interval as pulses.

The optimisation problem is solved analytically when the initial condition is stoichiometric. A sequential quadratic programming is used to determine the optimal policy when the initial condition is non-stoichiometric. We consider two cases i) when all sub-intervals are of equal duration ii) when the optimum duration of each sub-interval has to be found. The effect of initial conditions on the optimal profile is also investigated. We consider four possible modes of the operation of the reactor when (i) the final volume is fixed, final time is fixed, (ii) the final volume is free, final time is fixed, (iii) the final volume is fixed, final time is free, (iv) the final volume is free, final time is free.

# CHAPTER 1

## INTRODUCTION

Bio-chemical reactions characterised by substrate inhibition exhibit an optimum performance when the reactor is operated in the fed-batch mode. Here the substrate is fed to the reactor over a period of time to ensure that the reaction rate is close to the maximum in the reactor (Cazzador (1988), San and Stephanopolous (1984)).

Pontryagin's (1977) maximum principle has been used to solve this optimal control problem. Since the control variable, the substrate feed-rate occurs linearly in the system, the optimal policy is bang-bang and/or follows a singular trajectory. The control variable is maintained at its upper or lower bound, in bang-bang control, while along the singular arc the control variable varies continuously between the lower and upper bound.

Cazzador (1988) has studied the optimal feed rate profiles for fed batch fermentation that maximises the biomass production and the effect of initial conditions.

Fishman and Biryukov (1974) considered the problem of optimal glucose feed strategy for pencillin production. However, they did not give a numerical scheme, and ignored volume changes arising from the addition of the feed.

Weigand et al. (1981) reduced the problem of optimal feed rate determination for single cell protein production to one in which the total time of operation is minimised for a fixed rate of biomass production. This was analysed for the repeated fed-batch operation.

Park and Rogers have experimentally studied the factors which influence the growth kinetics of the L-phenylalanine strain of E-coli NST74. From the batch culture data it was observed that the increased initial glucose levels and increased L-phenylalanine product concentration caused a decrease in the specific rates of growth and rate of product formation, thereby reducing the yields. In fed-batch culture a relatively high L-phenylalanine concentration was achieved.

The application of the continuous maximum principle though elegant possesses many numerical problems. For problems linear in the control variable, the control action is divided into several parts - when the variable is at its upper bound, when it is at its lower bound or when it varies continuously. The convergence of the numerical scheme usually needs a priori information about the exact sequence of the different control actions. This is usually obtained using information from the physics of the problem (Modak (1986)).

In many problems however the interactions between the different variables can be very complex. Under these conditions it is not possible to decide on the exact sequence of control profiles which results in optimal performance. It is also experimentally difficult to maintain an optimal profile which varies continuously. In bio-chemical systems this problem is often avoided by adding the substrate as pulses at discrete instants of time.

Sargent et al. (1984) used a Sequential Quadratic Programming approach to determine the optimum feeding policy of a reactant in a series-parallel reaction sequence. They divided the entire

duration into different stages and obtained the flow rates required for optimum performance in each stage.

Biegler et al. (1984) have developed a technique based on Sequential Quadratic Programming and orthogonal collocation which enables us to evaluate the optimal variable efficiently.

Rosen and Luus (1991) have discussed the methods for calculating gradients for nonlinear optimal control problems for the rapid convergence.

In this work we discuss the approximation of the optimal policy by discretising the substrate-feed rate with pulses. The substrate is assumed to be added at discrete points of time. The reaction is assumed to have a Haldane dependency on the substrate concentration. When the initial conditions of the reactor are stoichiometric, the order of the equations can be reduced by one and this permits an analytical solution. For the case when the initial state is non-stoichiometric the system of equations has to be solved simultaneously. Here we have used a Sequential Quadratic Programming technique to obtain the pulse feed-rate policy for different number of sub-intervals.

The effect of the initial state of the reactor is determined for both these cases. The optimal operation is discussed for (i) the final volume is fixed, final time is fixed, (ii) the final volume is free, final time is fixed, (iii) the final volume is fixed, final time is free, (iv) the final volume is free, final time is free.

## CHAPTER 2

### Problem Formulation and Method of Solution

In our mode of operation the substrate is added instantaneously at discrete points of time. These time instants signal the beginning of each sub-interval. The entire cycle of reactor operation here is divided into different sub-intervals. In each sub-interval the reactor is operated in batch mode. The pulses which are added at the beginning of a sub-interval change the initial conditions for that sub-interval.

The entire time of operation  $[t_o, t_f]$  is divided into  $N$  different sub-intervals. The evolution in the  $i^{th}$  sub-interval  $(t_{i-1} < t < t_i)$  is governed by, the equations of the batch reactor

$$\dot{X} = \mu(S) \cdot X \quad 1(a)$$

$$\dot{S} = - \frac{\mu(S)}{Y} \cdot X \quad 1(b)$$

The equations represent the reactor state before the addition of the pulse at  $t_i$ . Here  $X$  and  $S$  represent the concentration of cell mass and substrate respectively and  $V$  is the volume of the reactor in the  $i^{th}$  interval and  $\mu(S)$  is specific rate of growth.

These evolution of the system is subject to initial condition. These conditions specify the state at the beginning i.e.  $t_o^-$ , or before the addition of the pulse at  $t_o^-$  as

$$X(t_o^-) = X_o \quad 2(a)$$

$$S(t_o^-) = S_o \quad 2(b)$$

$$V(t_o^-) = V_o \quad 2(c)$$

For the numerical work a substrate inhibition model is taken from the literature. The dependency of the specific growth rate on  $S$

is assumed to be given by

$$\mu(S) = \frac{\mu_m}{1 + \frac{Km}{S} + \frac{S}{K_i}}$$

From the mass balance equations at the time instants when the pulses are added, we have,

$$X(t_{i-1}^+) = \frac{V_{i-1}}{V_i} X(t_{i-1}^-) \quad 3(a)$$

$$S(t_{i-1}^+) = \frac{V_{i-1}}{V_i} S(t_{i-1}^-) + \frac{V_i - V_{i-1}}{V_i} S_F \quad 3(b)$$

where the subscript F stands for feed conditions. Here  $t_i^+$ ,  $t_i^-$  represent the instants immediately after and just before the addition of the pulse at  $t = t_i$ . The pulse is assumed to contain only substrate and no biomass. Our objective is to seek the pulses  $\Delta V_i$ 's to be added at  $t_i$ 's which will maximise the biomass concentration  $X(t_f)$ . A schematic indicating the different variables is depicted in Fig. 1.

### Methods of Solution:

We solve this problem using two techniques

- (i) Integral method
- (ii) Sequential Quadratic Programming (SQP)

**(i) Integral Method:** This technique can be used only when the initial conditions are in stoichiometric. The initial conditions are said to be stoichiometric when they satisfy

$$\frac{X(t_0^-)}{Y} + S(t_0^-) = S_F \quad 4(a)$$

After the addition of the first pulse ( $\Delta V_1$ ) the volume becomes

$$V_1 = V_0 + \Delta V_1 \quad 5(a)$$

The biomass and substrate concentrations after the pulse addition,

i.e., at  $t_0^+$ , are

$$X(t_0^+) = \frac{V_0}{V_1} X(t_0^-) \quad 5(b)$$

$$S(t_0^+) = \frac{V_0}{V_1} S(t_0^-) + \frac{V_1 - V_0}{V_1} S_F \quad 5(c)$$

Adding the two equations 5(b-c) we obtain

$$\begin{aligned} \frac{X(t_0^+)}{Y} + S(t_0^+) &= \frac{V_0}{V_1} \left[ \frac{X(t_0^-)}{Y} + S(t_0^-) \right] + S_F - \frac{V_0}{V_1} S_F \\ &= S_F \end{aligned}$$

This last identity follows from 4(a).

The evolution of the system (1a-b) during the batch is such that

$$\frac{d}{dt} \left( \frac{X}{Y} + S \right) = 0 \quad 6(a)$$

$$\text{or } \frac{X(t)}{Y} + S(t) = S_F \quad \text{for } t_{i-1} < t < t_i \quad 6(b)$$

clearly

$$\frac{X(t_i^-)}{Y} + S(t_i^-) = S_F = \frac{X(t_{i-1}^-)}{Y} + S(t_{i-1}^-) \quad 6(c)$$

Hence the variables  $X$ ,  $S$  satisfy the stoichiometric relation for all ' $t$ ' when the initial conditions at  $t_0^-$  are stoichiometric. This can be used to eliminate  $S$  in favour of  $X$  as

$$S = g(X) = S_F - \frac{X}{Y} \quad (7)$$

Then equation 1(a) and 2(b) can be reduced to the single equation

$$\dot{X} = \mu(g(X))X \quad (8)$$

Integrating this from  $t_0^+$  to  $t_1^-$ , we obtain

$$t = \int_{X(t_0^+)}^{X(t_1^-)} \frac{dX}{X\mu(g(X))} = t_1 - t_0 \quad 9(a)$$

Thus, the total final time of operation  $t_f$  we have



$$t_f = t_N - t_0 = \int_{X(t_0^+)}^{X(t_1^-)} \frac{dX}{X\mu(X)} + \int_{X(t_1^+)}^{X(t_2^-)} \frac{dX}{X\mu(X)} + \dots + \int_{X(t_{N-1}^+)}^{X(t_N^-)} \frac{dX}{X\mu(X)} \quad 9(b)$$

Using the jump discontinuity conditions 3(a,b) which arise due to the instantaneous pulses this yields

$$t_f = \int_{X(t_0^-)}^{X(t_1^+)} \frac{V_2}{V_1} \frac{dX}{X\mu(X)} + \int_{X(t_1^+)}^{X(t_2^+)} \frac{V_3}{V_2} \frac{dX}{X\mu(X)} \dots + \int_{X(t_{N-1}^+)}^{X_f} \frac{dX}{X\mu(X)} \quad (10)$$

The variables which are to be determined are (i) the volumes of the reactor,  $V_i$ , in each sub-interval  $i$  or alternatively the pulses to be added at  $t_i$ 's and (ii) the duration of each sub-interval. Alternatively we can evaluate the cell mass concentration at the end of each sub-interval,  $X(t_i^-)$ , instead of the duration of each sub-interval, since these uniquely determine the duration of each sub-interval. In the first part of the work we consider our manipulated variables to be  $X(t_i^+)$  and  $V_i$ 's

Our objective is to maximise the biomass concentration at the end of the cycle i.e.,  $X(t_f)$  or  $X_f$ . The subscript  $f$  is used to denote the final state of the reactor. The total number of variables to be determined are  $2N$ . These are  $N$ ,  $\Delta t_i$ 's, and  $N$ ,  $\Delta V_i$ 's

At the optimum conditions

$$\frac{\partial X_f}{\partial X(t_i^+)} = 0 \quad \text{for } i = 0 \quad 11(a)$$

$$\text{and } \frac{\partial X_f}{\partial V_i} = 0 \quad 11(b)$$

using these on (10) yields

$$\mu(X(t_i^+)) = \mu\left(X(t_i^+) \frac{V_{i+1}}{V_i}\right) \quad \text{for } i = 1, 2, \dots, (N-1) \quad 12(a)$$

$$\mu\left(\frac{X_0 V_0}{V_1}\right) = \mu\left(X(t_i^+) \frac{V_{i+1}}{V_i}\right) = \mu\left(X(t_{i+1}^+) \frac{V_{i+2}}{V_{i+1}}\right) \quad \text{for } i = 1, 2, \dots, (N-2) \quad 12(b)$$

Combining both (12a-b)

$$\mu\left(\frac{X_0 V_0}{V_1}\right) = \mu\left(X(t_i^+)\right) = \mu\left(X(t_i^+) \frac{V_{i+1}}{V_i}\right) = \mu\left(X(t_{i+1}^+) \frac{V_{i+2}}{V_{i+1}}\right) \quad 12(c)$$

for  $1 \leq i \leq N-1$

Writing it out for each,  $i$

$$\mu(X(t_1^+)) = \mu(X(t_2^+)) = \dots = \mu(X(t_{N-1}^+)) = \mu(X(t_0^+)) \quad 13(a)$$

$$\mu\left(X(t_1^+) \frac{V_2}{V_1}\right) = \mu\left(X(t_2^+) \frac{V_3}{V_2}\right) = \dots = \mu\left(X(t_{N-1}^+) \frac{V_f}{V_{N-1}}\right) \quad 13(b)$$

$= \mu(X(t_0^+))$

the possible solutions are

$$X(t_1^+) = X(t_2^+) = \dots = X(t_{N-1}^+) = \frac{X_0 V_0}{V_1} \quad 14(a)$$

$$X(t_1^+) \frac{V_2}{V_1} = X(t_2^+) \frac{V_3}{V_2} = \dots = \frac{X_0 V_0}{V_1} \quad 14(b)$$

Since the form of kinetic equation is such that there can be at most two values of  $X$ ,  $X_1$  ( $\neq X_2$ ) for which

$$\mu(X_1) = \mu(X_2),$$

A possible solution is

$$\frac{V_2}{V_1} = \frac{V_3}{V_2} = \dots = \frac{V_f}{V_{N-1}} = r \quad 15(a)$$

$$\text{and } X(t_1^+) = X(t_2^+) = \dots = X(t_{N-1}^+) = u \quad 15(b)$$

This ensures that we have N sub-intervals i.e. no sub-interval is of "zero" duration

$$t_f = \int_{\frac{x_o v_o}{v_1}}^{ru} \frac{dx}{x \mu(x)} + \int_u^{ru} \frac{dx}{x \mu(x)} + \dots + \int_u^{x_f} \frac{dx}{x \mu(x)} \quad 15(c)$$

$$v_1 = r^{1-1/N} v_f \quad 15(d)$$

$$t_f = \int_{\frac{x_o v_o}{r^{1-1/N} v_f}}^{ru} \frac{dx}{x \mu(x)} + (N-2) \int_u^{ru} \frac{dx}{x \mu(x)} + \dots + \int_u^{x_f} \frac{dx}{x \mu(x)} \quad 15(e)$$

Here the variables to be found are u, r. So we have for optimal conditions

$$\frac{\partial x_f}{\partial u} = 0 \text{ and } \frac{\partial x_f}{\partial r} = 0$$

This yields

$$\mu(u) = \mu(ru) \quad 16(a)$$

$$\mu(ru) = \mu\left(\frac{x_o v_o}{r^{1-1/N} v_f}\right) \quad 16(b)$$

combining (16a-b), since we do not want the first-interval to be of zero duration

$$u = \left(\frac{x_o v_o}{r^{1-1/N} v_f}\right) \quad 16(c)$$

$$\left(s_F - \frac{u}{Y}\right) \left(s_F - \frac{ru}{Y}\right) = K_m K_i \quad 16(d)]$$

The three equations 15(e), 16(c), 16(d) are solved simultaneously for a fixed  $v_f$ ,  $t_f$  to obtain r, u,  $x_f$  under the optimal conditions.

## (ii) Sequential Quadratic Programming (SQP):

The integral method proposed can not be applied when the initial conditions are non-stoichiometric. This case can be analysed using sequential quadratic programming. We discuss the solution methodology for two cases

- (i) When the duration is divided N equal sub-intervals.
- (ii) When the duration of each sub-interval is not constrained to be equal.

### (i) Equal Intervals:

Here in fed-batch mode of operation, we divide the duration of the operation into n equal time intervals. The substrate feed is added at the beginning of each sub-interval. The volume of the reactor is constant in each interval. The optimisation problem is to determine the optimal feed pulses to maximise final cell mass concentration ( $X_f$ ). We assume cell mass growth to be limited by one substrate as done earlier. Here the duration of each sub-interval is known <sup>since</sup> the total cycle time is fixed.

The differential equations which govern the system evolution are (1a-b). Constraints are imposed on the final reactor volume at the final time ( $t_f$ ),

$$V(t_f) = V_f \quad (17)$$

The updating conditions at the beginning of each interval are the conditions (3a-b).

In this approach the different charges  $\Delta V_i$ 's to be found for the optimal performance are chosen to satisfy the constraint. The numerical method used here is Sequential Quadratic Programming (SQP). The convergence of this method needs the accurate

determination of derivatives of the objective function with respect to manipulated variables, in this case pulses ( $\Delta V_i$ ).

The success of this method depends on the accurate computation of these derivatives. We have used state trajectory sensitivity method proposed by Rosen and Luus, 1991 to calculate the derivatives for faster convergence.

### Calculation of sensitivity with respect to $\Delta V_i$ 's:

For the case of equal sub-intervals we recall that our objective function is  $X_f$  and that the manipulated variables are  $\Delta V_i$ 's for  $i = 1, \dots, N$

Differentiating 1a-b with respect to  $\Delta V_j$ , we have for  $j = 1, \dots, N$

$$\frac{\partial \dot{X}}{\partial \Delta V_j} = \frac{\partial \dot{X}}{\partial X} \frac{\partial X}{\partial \Delta V_j} + \frac{\partial \dot{X}}{\partial S} \frac{\partial S}{\partial \Delta V_j}$$

$$\frac{\partial \dot{S}}{\partial \Delta V_j} = \frac{\partial \dot{S}}{\partial X} \frac{\partial X}{\partial \Delta V_j} + \frac{\partial \dot{S}}{\partial S} \frac{\partial S}{\partial \Delta V_j}$$

$$\frac{d}{dt} \left( \frac{\partial X}{\partial \Delta V_j} \right) = \mu(S) \frac{\partial X}{\partial \Delta V_j} + \frac{X(\mu(S))^2}{\mu_m} \left[ \frac{-K_m}{S^2} + \frac{1}{K_i} \right] \frac{\partial S}{\partial \Delta V_j} \quad 18(a)$$

similarly

$$\frac{d}{dt} \left( \frac{\partial S}{\partial \Delta V_j} \right) = \frac{-\mu(S)}{Y} \frac{\partial X}{\partial \Delta V_j} - \frac{X(\mu(S))^2}{Y\mu_m} \left[ \frac{-K_m}{S^2} + \frac{1}{K_i} \right] \frac{\partial S}{\partial \Delta V_j}$$

$$j = 1, \dots, n, t_{j-1} < t < t_j \quad 18(b)$$

The four equations (1a-b, 18a-b) are to be solved simultaneously from  $t_{j-1}^+$  to  $t_f$  where  $j = 1, \dots, n$ . The following updating conditions

for  $j = 1, \dots, N$  are valid for the sensitivity functions

$$\frac{\partial X}{\partial \Delta V_j} (t_{j-1}^+) = -X(t_{j-1}^-) \frac{V_{j-1}}{V_j^2}$$

$$\frac{\partial S}{\partial \Delta V_j} (t_{j-1}^+) = + (S_F - S(t_{j-1}^-)) \frac{V_{j-1}}{V_j^2},$$

when  $j > 1$  we should integrate only two equations 1(a-b) from  $t_0$  to  $t_{j-1}^-$

for  $j = 1 \dots N-1$

$i = j+1, \dots, N$

$$\frac{\partial X}{\partial \Delta V_j} (t_{i-1}^+) = X(t_{i-1}^-) \frac{V_i - V_{i-1}}{V_i^2} + \frac{V_{i-1}}{V_i} \frac{\partial X}{\partial \Delta V_j} (t_{i-1}^-)$$

$$\frac{\partial S}{\partial \Delta V_j} (t_{i-1}^+) = \frac{(S(t_{i-1}^-) - S_F) (V_i - V_{i-1})}{V_i^2} + \frac{V_{i-1}}{V_i} \frac{\partial S}{\partial \Delta V_j} (t_{i-1}^-)$$

Integrating these equations from  $t_j^+$  to  $t_N$  yields the  $\frac{\partial X(t_f)}{\partial \Delta V_j}$

This will give the gradients of objective function with respect to manipulated variables, (the pulses).

### (ii) Unequal Intervals:

In this approximation we divide the duration into  $N$  different intervals. Here we have  $2N$  variables to be found for optimal performance. Our manipulated variables are the  $\Delta V_i$ 's and  $\Delta t_i$ 's. For this case we have to find the derivatives with respect to  $\Delta V_i$ 's as well as  $\Delta t_i$ 's. The calculation of gradients with respect to  $\Delta V_i$ 's are the same as discussed above. The calculation of gradients with respect to  $\Delta t_i$ 's as follows.

Differentiating 1a-b with respect to  $\Delta V_j$ , we have

$$\frac{\partial \dot{X}}{\partial \Delta t_j} = \frac{\partial \dot{X}}{\partial X} \frac{\partial X}{\partial \Delta t_j} + \frac{\partial \dot{X}}{\partial S} \frac{\partial S}{\partial \Delta t_j}$$

$$\frac{\partial \dot{S}}{\partial \Delta t_j} = \frac{\partial \dot{S}}{\partial X} \frac{\partial X}{\partial \Delta t_j} + \frac{\partial \dot{S}}{\partial S} \frac{\partial S}{\partial \Delta t_j}$$

$$\frac{\partial \dot{X}}{\partial \Delta t_j} = \mu(S) \frac{\partial X}{\partial \Delta t_j} + \frac{X(\mu(S))^2}{\mu_m} \left[ \frac{-K_m}{S^2} + \frac{1}{K_i} \right] \frac{\partial S}{\partial \Delta t_j} \quad 20(a)$$

similarly

$$\frac{d}{dt} \left( \frac{\partial S}{\partial \Delta t_j} \right) = \frac{-\mu(S)}{Y} \frac{\partial X}{\partial \Delta t_j} + \frac{X(\mu(S))^2}{Y\mu_m} \left[ \frac{-K_m}{S^2} + \frac{1}{K_i} \right] \frac{\partial S}{\partial \Delta t_j}$$

$$j = 1, \dots, n, t_{j-1} < t < t_j \quad 20(b)$$

The four equations 1(a-b), 20(a-b), are to be simultaneously integrated from  $t_0^+$  to  $t_f$ . We can do it otherwise eliminating unnecessary computations.

Suppose if we differentiate with respect to  $j^{\text{th}}$  time interval then integrate the equations from  $t_j^+$  to  $t_f$ . Because

$$\left. \frac{\partial X}{\partial \Delta t_j} \right|_{t=t_j^-} = \left. \frac{dX}{dt} \right|_{t=t_j^-}$$

$$\left. \frac{\partial S}{\partial \Delta t_j} \right|_{t=t_j^-} = \left. \frac{dS}{dt} \right|_{t=t_j^-}$$

The updating conditions are

$$\frac{\partial X}{\partial \Delta t_j} (t_{j-1}^+) = \frac{V_{j-1}}{V_j} \frac{\partial X}{\partial \Delta t_j} (t_{j-1}^-)$$

$$\frac{\partial S}{\partial \Delta t_j} (t_{j-1}^+) = \frac{V_{j-1}}{V_j} \frac{\partial S}{\partial \Delta t_j} (t_{j-1}^-)$$

The effect of initial conditions on the optimal feed profile and the objective function is also tabulated.

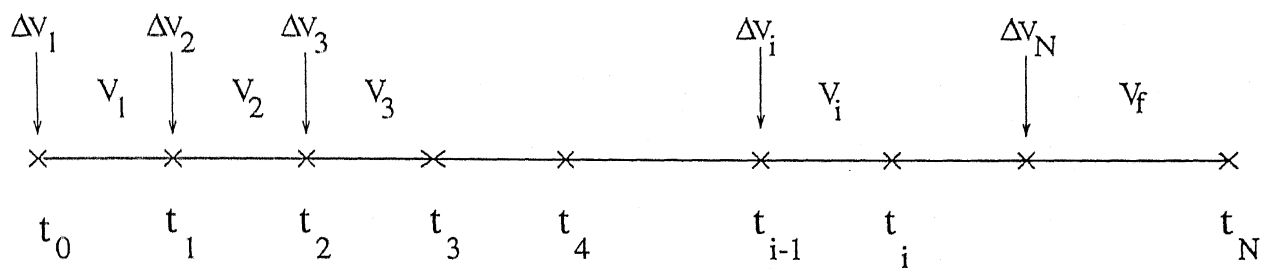


Fig.1. The variables in the different stages of a cycle.



## CHAPTER 3

### Result and Discussions

In this section we discuss the results of the computations obtained using the methods discussed earlier. We divide this section into two parts. Part 1, consists of results obtained using the integral method, non-stoichiometric equal and unequal intervals for the case of fixed  $t_f$  and fixed  $V_f$ . Part 2 deals with the results obtained for remaining three modes of reactor operations.

In our approach we have described the entire period of operation into 'N' sub-intervals. The volume of the reactor is increased from  $V_0$  to  $V_f$  by adding pulses  $\Delta V_i$  at the beginning of the  $i^{\text{th}}$  sub-interval. For the case of equal time intervals the problem reduces to determining the N-variables,  $\Delta V_i$ 's to maximise  $X_f$ .

For the case of unequal sub-intervals, we have to obtain 2N variables, the pulses to be added at the beginning of the sub-intervals, as well as their durations.

#### PART 1:

The integral method can be used only when the biomass 'X' and the substrate concentration 'S' satisfy the stoichiometric relationship. This enables us to reduce the two dimensional system to a one-dimensional system. The optimal profile of the pulses is obtained by the simultaneous solution of the three equations 15(e), 16(c), 16(d) for  $u$ ,  $r$  and  $X_f$ . The parameters chosen for simulation are  $K_m = 0.397458$ ,  $K_i = 62.9$ ,

$\mu_m = 0.347695$ ,  $Y = 0.2$ ,  $S_F = 10$ ,  $X_O = 1.0$ ,  $V_O = 1.0$ ,  $V_f = 2.5$ ,  
 $T_f = 5$ .

In Table-1 we depict the numerical results of the computations for different values of 'N', the number of sub-intervals. The first (N-1) sub-intervals are of equal duration while the last sub-interval is distinct from these. We have also depicted the corresponding  $u$  - the biomass concentration,  $r$  - the ratio of the reactor volumes of successive intervals i.e.,  $V_{i+1}/V_i$ , ( $i = 1, \dots, n-1$ ), and  $X_f$  the biomass at the end of the duration.

The biomass concentration  $X_f$  increases with the number of pulses. This confirms our expectations as increasing N results in obtaining a better approximation. In the batch mode the biomass concentration is 1.7537.

The improvement in the performance when operated in the fed-batch mode in this case is marginal. For the choice of parameters listed here the fed-batch reactor gives only a 0.542% improvement over the batch reactor for N=10. Fig. 2a depicts the variation of reactor volume for N=5. The corresponding variation of  $X$  is shown in Fig. 2b. The addition of the pulses results in discontinuities in the biomass concentrations.

The integral method proposed here has some inherent limitations. The method can converge to infeasible solutions. Thus although  $r > 1$ ,  $u > 0$  and  $X_f > 0$ ,  $V_1 = V_f r^{1-n}$ , may be less than  $V_O$ . This solution is not feasible since it yields  $V_1 < V_O$ . Such solutions do satisfy the equations but however are physically meaningless.

A tacit assumption in the integral method is that we add pulses at the beginning of each sub-interval. Clearly a solution to the equations is that some of the pulses are zero, i.e.,  $r = 1$ , for some cases, while for others  $r > 1$ . We have not considered this case. This method also implies that the duration of a sub-interval is zero when  $r = 1$ . It does not consider the possibility of reactor operation when the  $i^{\text{th}}$  pulse can be zero, and the duration of the  $i^{\text{th}}$  sub-interval is non-zero.

The optimum value reported as in Table-1 is hence subject to these tacit assumptions.

### Sequential Quadratic Programming:

For the case when the initial conditions do not satisfy the stoichiometric relationship we can not use the integral method discussed above since now the problem is two dimensional. We have solved this problem using Sequential Quadratic Programming. The function 'CONSTR' available in MATLAB <sup>was</sup> used to solve this problem.

### Equal Sub-intervals:

In this method all sub-intervals are considered to be of equal durations. We consider the operation where the final reactor volume and the duration of the entire cycle are both externally fixed. The duration of each sub-interval is hence determined once we fix 'N'. Hence in this problem one has to determine only the N-pulses, i.e.,  $\Delta V_i$ 's which maximises  $X(t_f)$ .

In this mode of reactor operation the optimal profile will be dependent on the initial state of the reactor. There are three characteristic quantities which characterize the substrate

concentration,  $S_0$ , the initial substrate concentration,  $S_F$  the feed concentration,  $S^*$  the concentration where  $\mu(S)$  has a maximum.

The relative ordering of the different quantities gives rise to different cases. These have been tabulated in Table 2. The performance of the reactor i.e., the sequence of the pulses for each of these cases is tabulated in Table 3 for fixed  $V_f = 10$ ,  $t_f = 30$  and  $N=5$ .

We now discuss the trends of the optimal profile obtained in Table 3, for the different conditions.

**Case I:**  $S_0 < S^* < S_F$ : The initial concentration is lower than the  $S^*$ . Since the reaction rate is maximum at  $S^*$ , the performance is optimal when the substrate concentration is maintained closer to  $S^*$ . It is hence necessary to add the first pulse to increase  $S_0$  to  $S^*$ . The optimal pulse distribution which satisfies all constraints is shown in Table 3. The optimal  $X_f$  here is 2.8372. For this case the batch operation has an  $X_f = 1.6012$ . Fig. 3a depicts the volume of the reactor after the addition of each pulse. The volume changes discontinuously at the point of addition of each pulse. The corresponding biomass and substrate concentrations are shown in Fig. 3b(i) and Fig. 3b(ii).

**Case II:**  $S_F < S^* < S_0$ : In case 2a,  $S_0$  is approximately  $S^*$  and  $S_F$  is very low. Addition of  $S_F$  will hence hurt the reactor performance. The pulse addition is hence delayed till the end. This addition is necessary since we have to satisfy the volume constraint. For case 2b,  $S_0$  is significantly higher than  $S^*$ . Addition of  $S_F$  reduces  $S_0$  to  $S^*$  now and this yields the optimal profile shown. Here the fed-batch performance is same as batch.

**Case III:**  $S_O < S_F < S^*$ : Since the initial concentration is low addition of the feed increases the substrate concentration close to  $S^*$ . Here it is advantageous to add all the pulses in the first sub-intervals as this brings  $S_O$  closest to  $S^*$ . In this case the final biomass concentration is same for fed-batch and batch reactors.

**Case IV:**  $S^* < S_F < S_O$ : Here the first pulse is added as in case III, at the first sub-interval. This brings  $S_O$  closest to  $S^*$  and the reaction rate is closest to the maximum. Here again the performance of the fed-batch and batch reactors are identical.

**Case V:**  $S^* < S_O < S_F$ : Here the initial concentration is closer to  $S^*$  than  $S_F$ . Addition of the feed would thus increase  $S_O$  and lower the reaction rate. This addition should be hence delayed as far as possible. This confirms the trend shown in the table.

**Case VI:**  $S_F < S_O < S^*$ : Here again addition of the feed would drive the concentration away from  $S^*$ , lowering the reaction rate. Hence this should be delayed. In this case the substrate is added in the last interval to satisfy the volume constraint.

#### **Unequal Sub-intervals (Fixed $t_f$ and Fixed $V_f$ ):**

In Table-4 we depict the results of the computations for the case of unequal time intervals. Here  $t_f = 30$ , and  $V_f = 10$ . We have presented the results for the same  $N$  as in Table-3. This enable us to compare the two results.

The qualitative trends of the two Tables agree well. The performance of each case for a given ' $N$ ' is better when the intervals are of unequal duration as compared to when we

restrained the intervals to be equal.

We simulated the behaviour of the system for the case of stoichiometric initial conditions with SQP algorithm for unequal sub-intervals. The parameters set chosen is the one used to generate the results using the integral method. The SQP method also yields the same optimal profile as the integral method. The length of  $(N-1)$  sub-intervals is equal and the last one is distinct. The successive volumes  $V_{i+1}/V_i$  also bear a constant ratio for  $i=1,2,\dots,N$ . The optimal objective function also matches to within a desired numerical accuracy with the results in (Table-1).

## PART 2:

We now discuss the application of this method to remaining three modes of reactor operation (i) fixed final time, free final volume (ii) free final time and fixed final volume (iii) free final time, and free final volume.

The optimisation problem solved so far have some cases where the volume of the reactor is made up in the last sub-interval. This is forced on the system since it has to satisfy the constraint on the reaction volume. It would hence appear that should this constraint be removed the optimal profile be one in which no pulses are added. To test this hypothesis removed the constraint on the reaction volume. We now discuss the results for the situation when the reactor is operated over a time  $t_f$  and the final free volume  $V_f$ .

For cases 2, 4, 6 of Table-5, here, there is no pulse added for optimal performance. The optimal performance is in batch mode with reactor volume equal to the initial volume. hence the final pulse added for the fixed volume problems, in Table-4 is only to satisfy the constraint of the fixed final volume.

For cases 1, 5 pulses are added in each sub-intervals. The final volume for both the cases is less than 10. In case 1:  $V_f = 7.96$ , while for case 5 it is 7.1925. The corresponding cases in Table-4, also have non-zero pulses being added in each sub-interval.

For case 3, the optimal performance is found when the initial pulse is non-zero as in Table-3. For this case the optimisation is carried out by constraining the final volume  $V_f$  to be lower than the upper bound. The upper bound chosen for this case study was

20. The reactor is filled in the first pulse, since this raises  $S_0$  closer to  $S^*$ . This is again identical to the optimal profile in Table-4.

One would expect a similar trend for case 4, i.e., addition in the first pulse to being  $S_0$  to  $S^*$ . However, for the values chosen  $S_0$ ,  $S_F$  are very closer to  $S^*$  and hence batch mode at  $V_0$  is optimal.

The improvement of the performance as measured by the concentrations  $X_f$  for each case in Table-5 is much better than for the corresponding cases in Table-4. The free volume problem has a better performance than the reactor with a fixed final volume.

The optimal performance for a given number of pulses may not be necessarily obtained when the sub-intervals are of the same duration. The constraint of each sub-intervals results in obtaining a sub-optimal profile for a given  $N$ .

To obtain a time optimal profile for a fixed number of intervals  $N$ , we now discuss the results obtained when the sub-intervals are of unequal durations. Now for a given number of  $N$  sub-intervals we have to determine  $2N$  variables, the  $N \Delta t_i$ 's and the  $N \Delta V_i$ 's. We solve this problem also using "CONSTR" of MATLAB. The convergence of the algorithm is based on the accurate calculation of the sensitivities of the objective function with respect to the control variables. These are computed as discussed earlier.

In Table-6, we depict the results for fixed  $t_f$  and free  $V_f$ , with unequal sub-intervals. The effect of initial conditions on the optimal profile is similar to that predicted in Table-5. For each case the optimal solution is better when we relax the



constraints of equal sub-intervals as expected.

Table-7, contains the results for  $V_f = 10$ , and free  $t_f$ . The solution method requires an upper bound on free time. This was fixed at 150. In most of the cases as shown the optimal profile was found such that the cycle time was 150. A characteristic feature of most of the runs is that the system possesses multiple optima.

The convergence to the different optima depends on the initial guesses supplied. Although the profile can be very different there is no change in the objective function. For this mode the improvement in the objective function over the batch mode is negligible in most cases. This occurs because the system tends to choose the largest possible time to allow the reaction to go to completion. This results in maximum biomass.

Table-8 depicts the results for the free  $V_f$  and free  $t_f$  problem. Here again we have to impose upper bounds on these variables to the optimal profile obtained in most cases is when the reactor is operated in the batch mode. The choice of  $t_f$ ,  $V_f$  here is such that the biomass  $X_f$  at the end of the cycle is much more than that obtained in the earlier modes of operation for each combination of  $S_0$ ,  $S^*$ ,  $S_F$ . The corresponding biomass and substrate concentrations for unequal intervals are shown in Fig.4b(i) and Fig.4b(ii).

Table-9 contains the results for  $V_f = 10$ , and free  $t_f$  for equal intervals of time. We impose an upperbound on free time. This was fixed at 2500. The convergence to the different optima depends on the initial guesses supplied. Here also the performance of fed-batch reactor is almost equal to the batch

mode.

Table-10 depicts the results for free  $V_f$  and free  $t_f$  problem. Here we impose an upper bound on these variables. The optimal profile obtained in most cases is when the reactor is operated in the batch mode.

## CHAPTER 4

### Conclusions

Here we have demonstrated different methods which can be used to obtain the optimal performance of fed-batch reactions. The flow rate over the entire cycle is approximated by discretised pulses.

The integral method is applicable only when the initial conditions are stoichiometric. It is also tacitly assumes that all pulses are non-zero.

Sequential quadratic programming allows us to obtain optimal profiles for the case of non-stoichiometric initial conditions. The effect of  $S_O$ ,  $S_F$ ,  $S^*$  has been studied and explained qualitatively.

The numerical convergence of SQP algorithm requires the accurate computation of the sensitivities. An efficient way to evaluate these is discussed.

For a particular set of parameters the case of stoichiometric unequal intervals yields the best possible optimal solution. The SQP method for the stoichiometric case can yield optimal profile even when the integral method gives infeasible solution.

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Table 1

## Integral Method

N	$(N-1)\Delta t$ 's	$\Delta t_n$	u	r	$X_f$
batch (N=1)	0	5.0	-	-	1.7537
3	1.1818	2.6363	0.8125	1.4252	1.7623
5	0.6714	2.3142	0.8952	1.2231	1.763
7	0.4675	2.1954	0.9277	1.1505	1.7632
10	0.3207	2.1136	0.9508	1.1010	1.7632

Table 2

Parameters used for Table (3-10) are  $\mu_m = 0.208$ ,  $K_m = 2.516$ ,  
 $K_i = 25$ ,  $Y = 0.8$ ,  $X_o = 0.1$ ,  $v_o = 7$ ,  $S^* = 7.93095$

Case	$S_o$	$S_F$	Description
1	$0.142857 \times 10^{-3}$	75	$S_o < S^* < S_F$
2a	8	$0.142857 \times 10^{-3}$	$S_F < S^* < S_o$
2b	20	4	$S_F < S^* < S_o$
3	$0.142857 \times 10^{-3}$	5.0	$S_o < S_F < S^*$
4a	8	7.95	$S^* < S_F < S_o$
4b	20	10	$S^* < S_F < S_o$
5a	7.95	75	$S^* < S_o < S_F$
5b	10	75	$S^* < S_o < S_F$
6	3.0	1.0	$S_F < S_o < S^*$

Table 3

Non-stoichiometric, Equal Intervals of Time, Fixed  $T_f$ , Fixed  $V_f$ 

Case	$\Delta V_1$	$\Delta V_2$	$\Delta V_3$	$\Delta V_4$	$\Delta V_5$	Fed-batch $X_f$	Batch $X_f$
1	0.8343	0.0226	0.0487	0.1057	1.9888	2.8372	1.6012
2a	-	-	-	-	3.0	2.7845	2.5542
2b	3.0	-	-	-	-	2.4624	2.4624
3	3.0	-	-	-	-	0.5493	0.5493
4a	1.5294	1.4706	-	-	-	3.0911	3.0911
4b	3.0	-	-	-	-	2.212	2.212
5a	0.0047	0.00224	0.0486	0.1064	2.818	2.6703	1.1918
5b	-	-	-	-	3.0	2.5857	1.11945
6	-	-	-	-	3.0	1.157	1.001



Table 4

Non-stoichiometric, Unequal Intervals Fixed  $T_f$  and Fixed  $V_f$ 

Case		i					Fed-batch $X_f$	Batch $X_f$
		1	2	3	4	5		
1	$\Delta V_i$	0.8577	0.1122	0.1518	0.1847	1.6926	3.1817	1.6012
	$\Delta t_i$	15.805	6.692	4.31	3.20	-		
2a	$\Delta V_i$	-	-	-	-	3	2.967	2.5542
	$\Delta t_i$	8.1178	8.1195	8.1195	5.6432	-		
2b	$\Delta V_i$	3.0	-	-	-	-	2.4624	2.4624
	$\Delta t_i$	5.578	5.9645	6.095	6.158	6.204		
3	$\Delta V_i$	3.0	-	-	-	-	0.5493	0.5493
	$\Delta t_i$	5.849	5.9744	5.975	6.036	6.170		
4a	$\Delta V_i$	0.762	0.761	0.76	0.717	-	3.0911	3.0911
	$\Delta t_i$	1.968	3.968	5.968	7.979	10.117		
4b	$\Delta V_i$	3.0	-	-	-	-	2.2120	2.2120
	$\Delta t_i$	7.64	7.78	7.78	6.81	-		
5a	$\Delta V_i$	0.028	0.1131	0.1518	0.1851	2.522		
	$\Delta t_i$	15.7815	6.697	4.315	3.21	-	3.1808	1.198
5b	$\Delta V_i$	-	-	-	0.2271	2.7729	3.1248	1.1195
	$\Delta t_i$	9.354	8.804	7.447	4.3956			
6	$\Delta V_i$	-	-	-	-	3.0		
	$\Delta t_i$	9.881	9.9806	10.081	0.0587	-	1.17895	1.0096

Table 5

Non-stoichiometric, Equal Intervals of Time, Fixed  $T_f$ , Free  $V_f$ 

Case	$\Delta V_1$	$\Delta V_2$	$\Delta V_3$	$\Delta V_4$	$\Delta V_5$	Fed-batch $X_f$	Batch $X_f$
1	0.8343	0.0226	0.0487	0.0953	0	3.92424	3.8938
2a	-	-	-	-	-	4.23861	4.23861
2b	-	-	-	-	-	2.6805	2.6805
3	10.094	-	-	-	-	0.8469	0.8469
4a	-	-	-	-	-	4.23861	4.23861
4b	-	-	-	-	-	2.6805	2.6805
5a	0.0046	0.0226	0.0487	0.1060	0.0106	4.36098	4.3077
5b	-	-	-	-	-	4.4159	4.4159
6	-	-	-	-	-	4.23861	4.23861

Table 6

Non-stoichiometric, Unequal Intervals Fixed  $T_f$  and Free  $V_f$ 

Case		i					Fed-batch $X_f$	Batch $X_f$
		1	2	3	4	5		
1	$\Delta V_i$	0.8419	0.0432	0.0547	0.0644	-	3.9245	3.8924
	$\Delta t_i$	10.312	5.4711	3.7455	5.3416	5.1308		
2a	$\Delta V_i$	-	-	-	-	-		
	$\Delta t_i$	5.806	5.9048	6.0029	6.0976	6.1876	4.2386	4.2386
2b	$\Delta V_i$	-	-	-	-	-		
	$\Delta t_i$	5.9989	5.9990	6.001	6.001	6.002	2.6805	2.6805
3	$\Delta V_i$	10.094	-	-	-	-	0.84696	0.84696
	$\Delta t_i$	2.5047	6.0501	6.8642	7.2006	7.3804		
4a	$\Delta V_i$	-	-	-	-	-	4.2386	4.2386
	$\Delta t_i$	5.801	5.901	6.001	6.101	6.1964		
4b	$\Delta V_i$	-	-	-	-	-		
	$\Delta t_i$	5.4717	5.8039	6.1004	6.2575	6.366	2.6805	2.6805
5a	$\Delta V_i$	0.0134	0.0482	0.0614	0.0727	-	4.36719	4.3057
	$\Delta t_i$	10.8834	5.6831	3.8212	3.7499	5.8624		
5b	$\Delta V_i$	-	-	-	-	-		
	$\Delta t_i$	7.673	7.673	6.226	4.9555	4.067	4.4159	4.4159
6	$\Delta V_i$	-	-	-	-	-		
	$\Delta t_i$	6.003	6.003	6.003	6.003	5.988	1.68421	1.68421

Table - 7

Unequal Intervals of Time, Fixed Final Volume, and Free Final Time

Case		i					Fed-batch	Batch
		1	2	3	4	5	$X_f$	$X_f$
1	$\Delta V_i$	0.2601	2.7399	-	-	-	18.07	18.07
	$\Delta t_i$	10.119	9.503	21.064	9.7196	10.689		
2a	$\Delta V_i$	-	-	-	0.8429	2.1571	4.55	4.55
	$\Delta t_i$	30.016	30.116	30.216	30.189	29.462		
2b	$\Delta V_i$	1.4175	0.6373	-	-	0.9452	12.23	12.23
	$\Delta t_i$	28.827	29.09	29.34	30.26	32.49		
3	$\Delta V_i$	3.0	-	-	-	-	1.2708	1.2708
	$\Delta t_i$	-	2.0399	3.91	52.72	91.34		
4a	$\Delta V_i$	1.5854	0.8037	0.204	0.204	0.204	6.458	6.458
	$\Delta t_i$	29.8	29.9	30.0	30.1	30.2		
4b	$\Delta V_i$	1.1024	0.2938	-	0.2059	1.3979	13.67	13.67
	$\Delta t_i$	29.511	29.356	29.554	30.2	31.73		
5a	$\Delta V_i$	0.7125	2.2875	-	-	-	22.522	22.522
	$\Delta t_i$	29.8195	23.05	3.953	-	1.264		
5b	$\Delta V_i$	3.0	-	-	-	-	23.67	23.67
	$\Delta t_i$	80.88	41.36	1.27	-	11.83		
6	$\Delta V_i$	1.6178	1.3822	-	-	-	1.99	1.99
	$\Delta t_i$	45.813	38.49	31.15	23.29	11.254		

Table - 8  
Unequal Intervals of Time, Free Final Volume, and Free Final Time

Case		i					Fed-batch $X_f$	Batch $X_f$
		1	2	3	4	5		
1	$\Delta V_i$	0.0154	1.8217	0.0113	1.2379	89.8138	55.807	11.76
	$\Delta t_i$	2.5	48.361	5.281	6.953	70.744		
2a	$\Delta V_i$	-	-	-	-	-	6.5	6.5
	$\Delta t_i$	16.37	16.47	16.57	16.67	16.77		
2b	$\Delta V_i$	-	-	-	-	-	16.1	16.1
	$\Delta t_i$	14.12	14.22	14.32	14.42	14.52		
3	$\Delta V_i$	146.685	0.6511	4.6065	14.098	26.8596	3.8635	3.8635
	$\Delta t_i$	161.36	162.77	163.77	164.77	166.1		
4a	$\Delta V_i$	-	-	-	-	-	8.1	8.1
	$\Delta t_i$	13.461	13.56	13.66	13.77	13.86		
4b	$\Delta V_i$	-	-	-	-	-	16.1	16.1
	$\Delta t_i$	15.45	16.45	17.45	18.45	19.43		
5a	$\Delta V_i$	0.6945	3.884	0.1977	0.7982	87.4255	56.2522	22.36
	$\Delta t_i$	29.0819	42.09	20.74	10.693	41.407		
5b	$\Delta V_i$	0.694	3.88	0.197	0.798	87.43	56.37	21.96
	$\Delta t_i$	29.1	42.1	20.7	10.69	41.0		
6	$\Delta V_i$	-	-	-	-	-	2.5	2.5
	$\Delta t_i$	45.0	38.0	31.0	23.0	11.0		

Table - 9

Equal Intervals of Time, Fixed Final Volume, and Free Final Time

Case	$\Delta t$	$\Delta V_1$	$\Delta V_2$	$\Delta V_3$	$\Delta V_4$	$\Delta V_5$	Fed-batch $X_f$	Batch $X_f$
1	56.45	3.0	-	-	-	-	18.0701	18.0701
2a	45.954	-	-	-	0.52	2.48	4.55	4.55
2b	17.098	1.8931	0.9501	0.1567	-	-	12.23	12.23
3	169.927	3.0	-	-	-	-	1.2701	1.2701
4a	192.236	0.0001	0.0004	0.0003	0.0003	2.9988	6.458	6.458
4b	14.684	-	0.4368	-	-	2.5632	13.67	13.67
5a	67.811	-	1.7762	1.2238	-	-	22.52	22.52
5b	55.474	3.0	-	-	-	-	23.67	23.67
6	44.691	0.186	1.8868	0.927	-	-	1.99	1.99

Table - 10

Equal Intervals of Time, Free Final Volume, and Free Final Time

Case	$\Delta t$	$\Delta V_1$	$\Delta V_2$	$\Delta V_3$	$\Delta V_4$	$\Delta V_5$	Fed-batch $X_f$	Batch $X_f$
1	30.0	4.82	-	5.41	9.4	73.4	55.81	52.27
2a	16.76	-	-	-	-	-	6.5	6.5
2b	29.1	-	-	-	-	-	16.1	16.1
3	29.97	93.0	-	-	-	-	3.727	3.7065
4a	16.06	-	-	-	-	-	6.5	6.5
4b	15.89	-	-	-	-	-	16.1	16.1
5a	29.77	42.022	0.004	0.005	1.536	49.43	56.252	33.416
5b	29.948	0.0663	2.1801	-	8.9635	81.7902	56.367	35.561
6	24.24	-	-	-	-	-	2.5	2.5

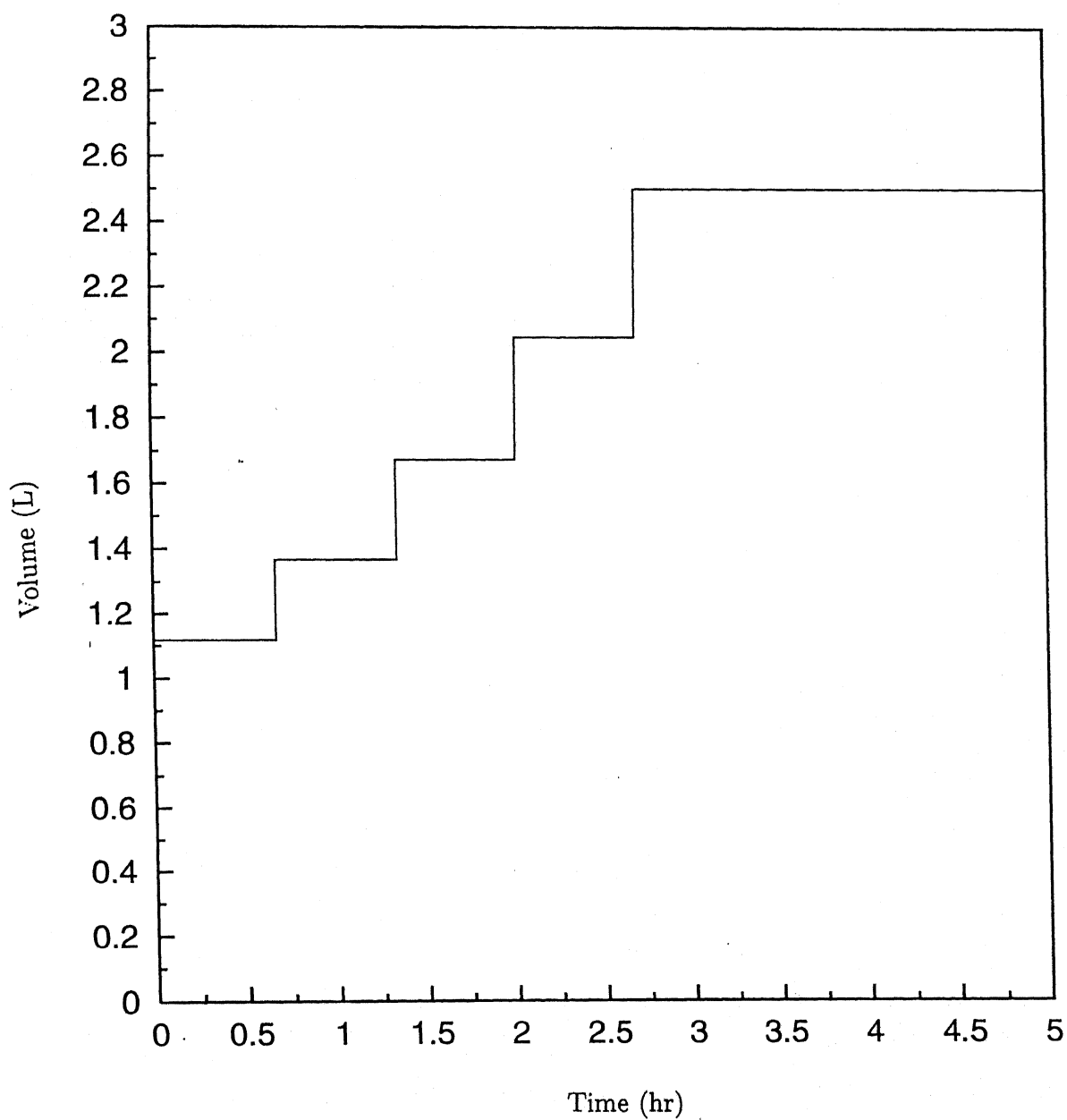


Fig. 2a : Volume profile for integral method N=5.



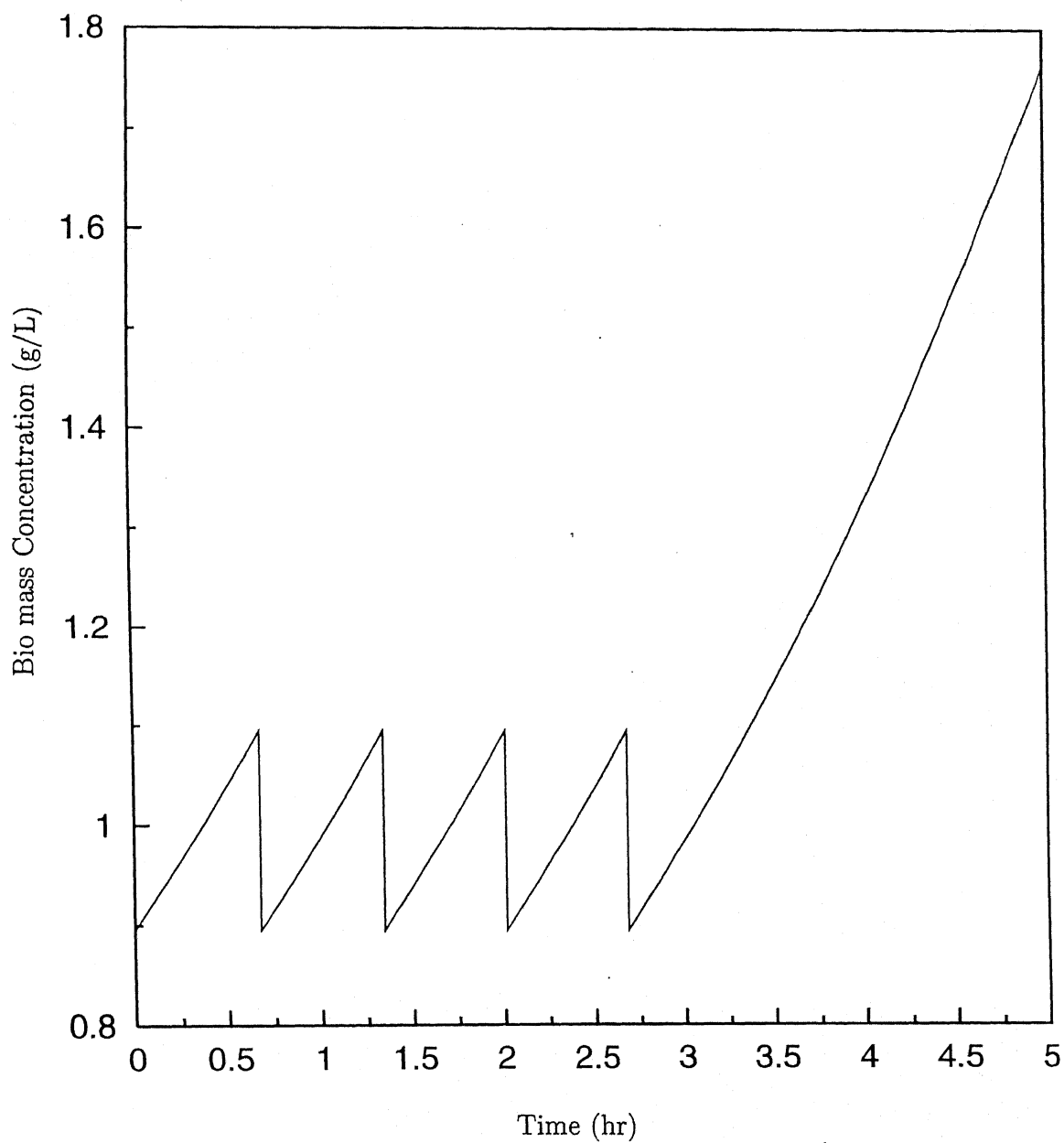


Fig. 2b : Biomass concentration profile for integral  
N=5

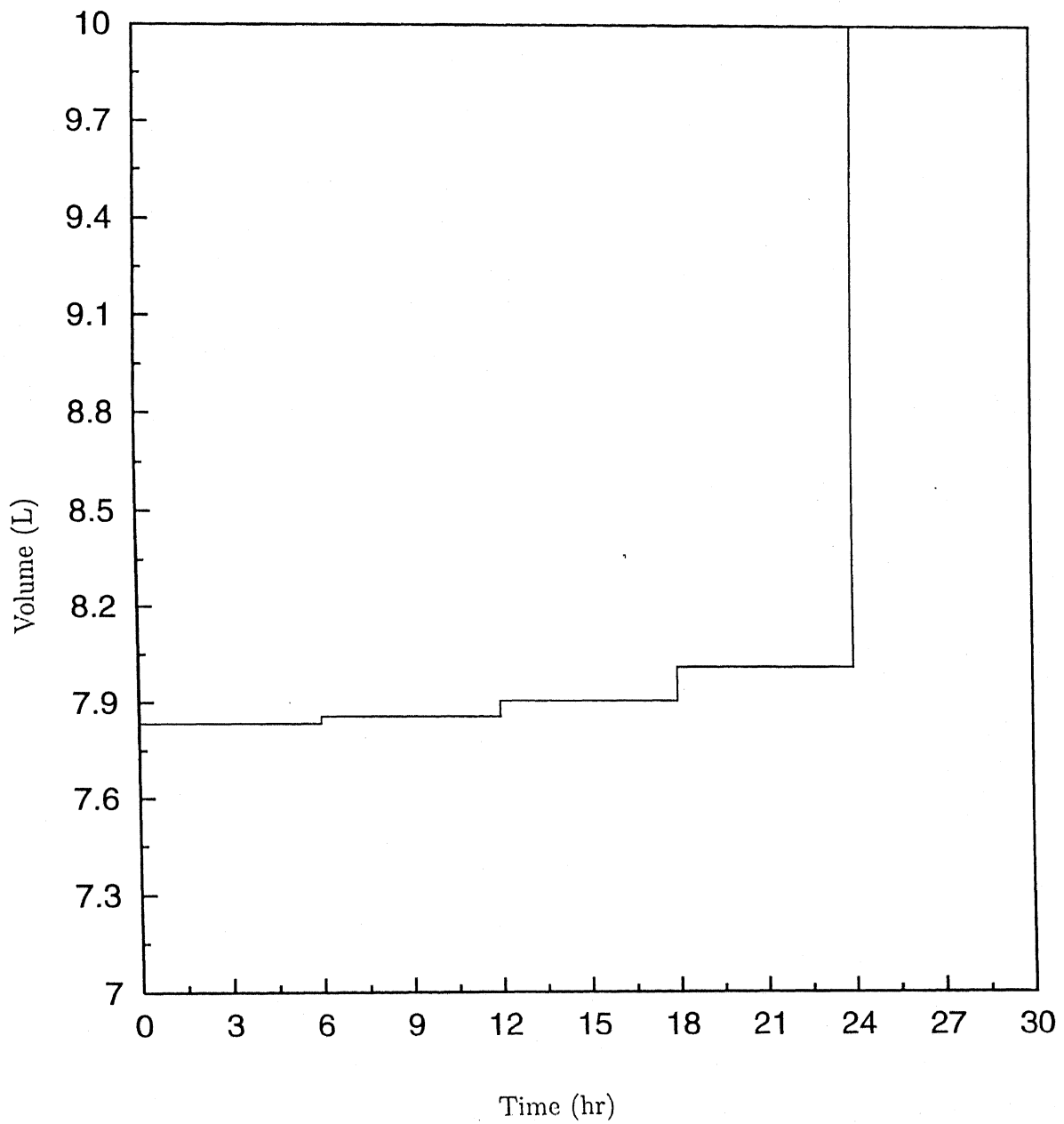


Fig. 3a : Volume profile for the fixed  $V_f$  and  $t_f$  with equal intervals.

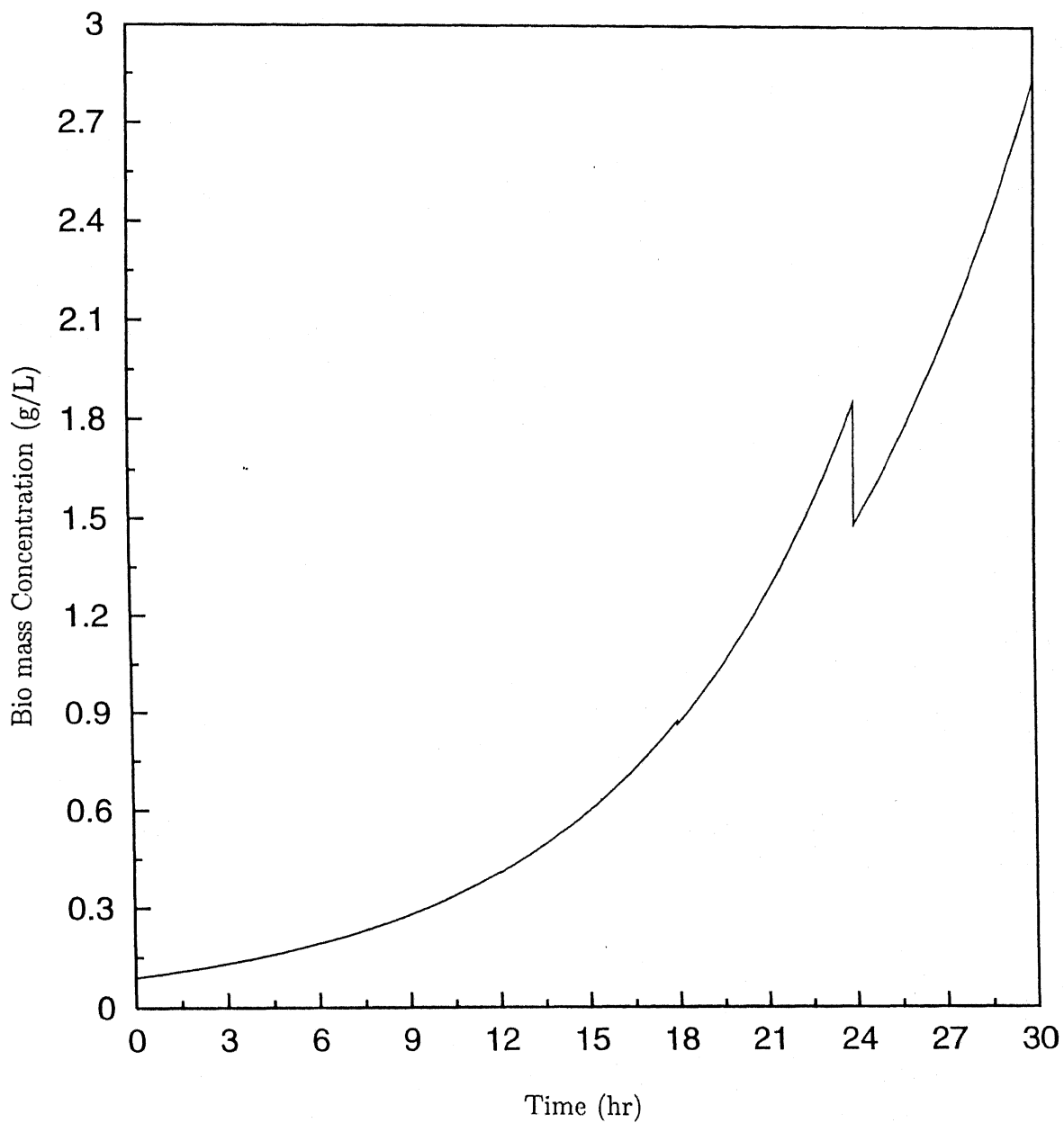


Fig. 3b(i) : Biomass concentration profile for the fixed  $V_f$  and  $t_f$  with equal intervals.

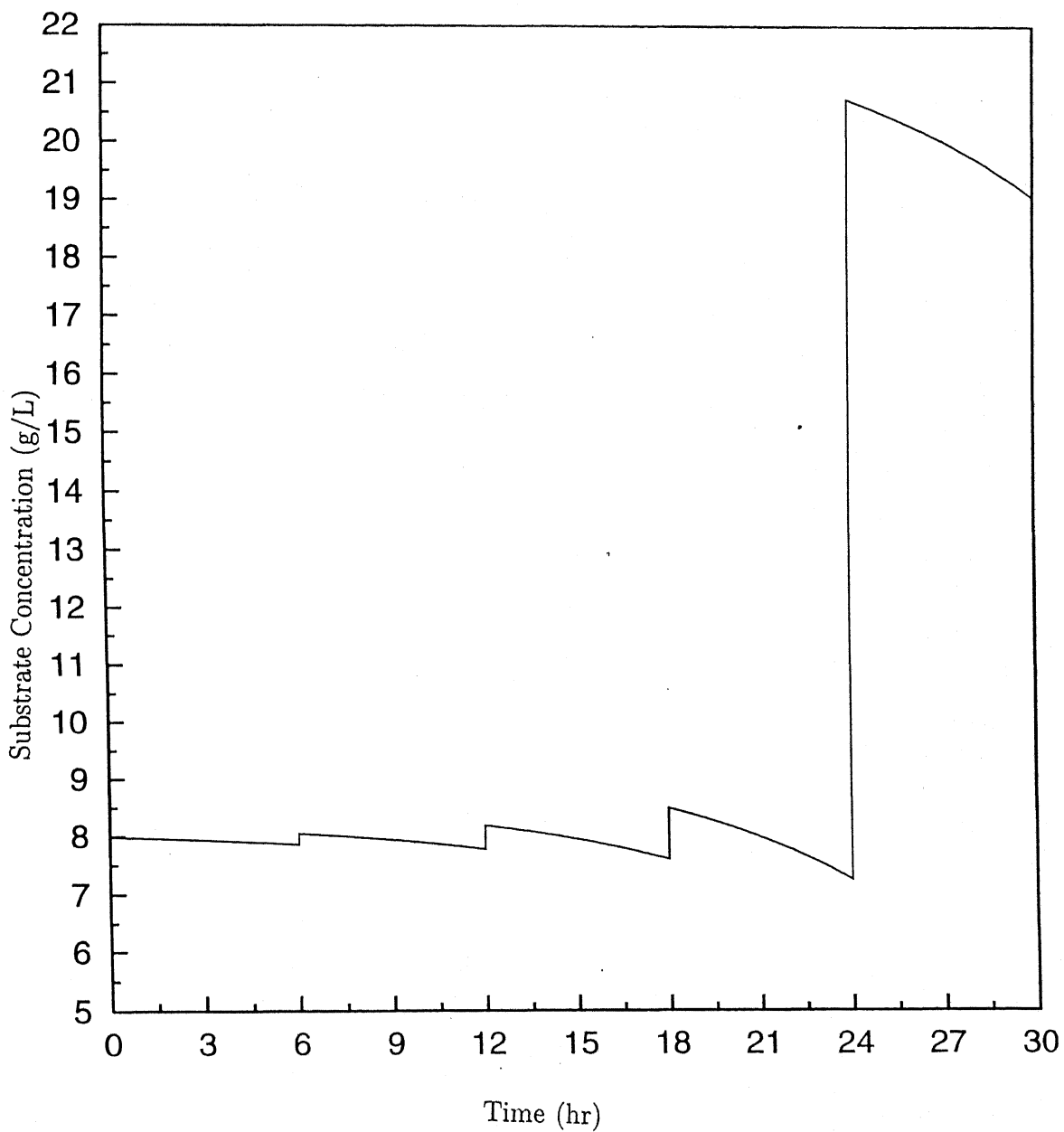


Fig. 3b(ii) : Substrate concentration profile for the fixed  $V_f$  with equal intervals.

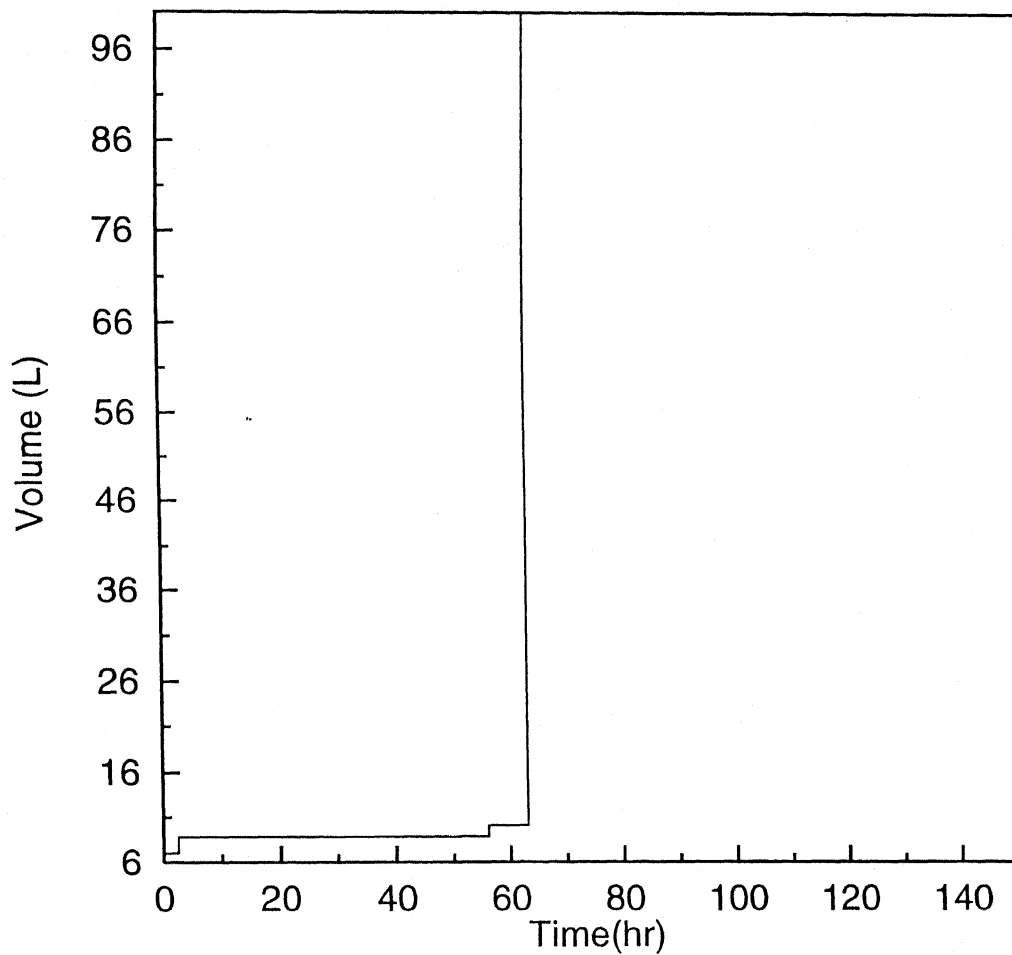


Fig. 4a : Volume profile for the Free  $V_f$  and  $t_f$  with unequal intervals.

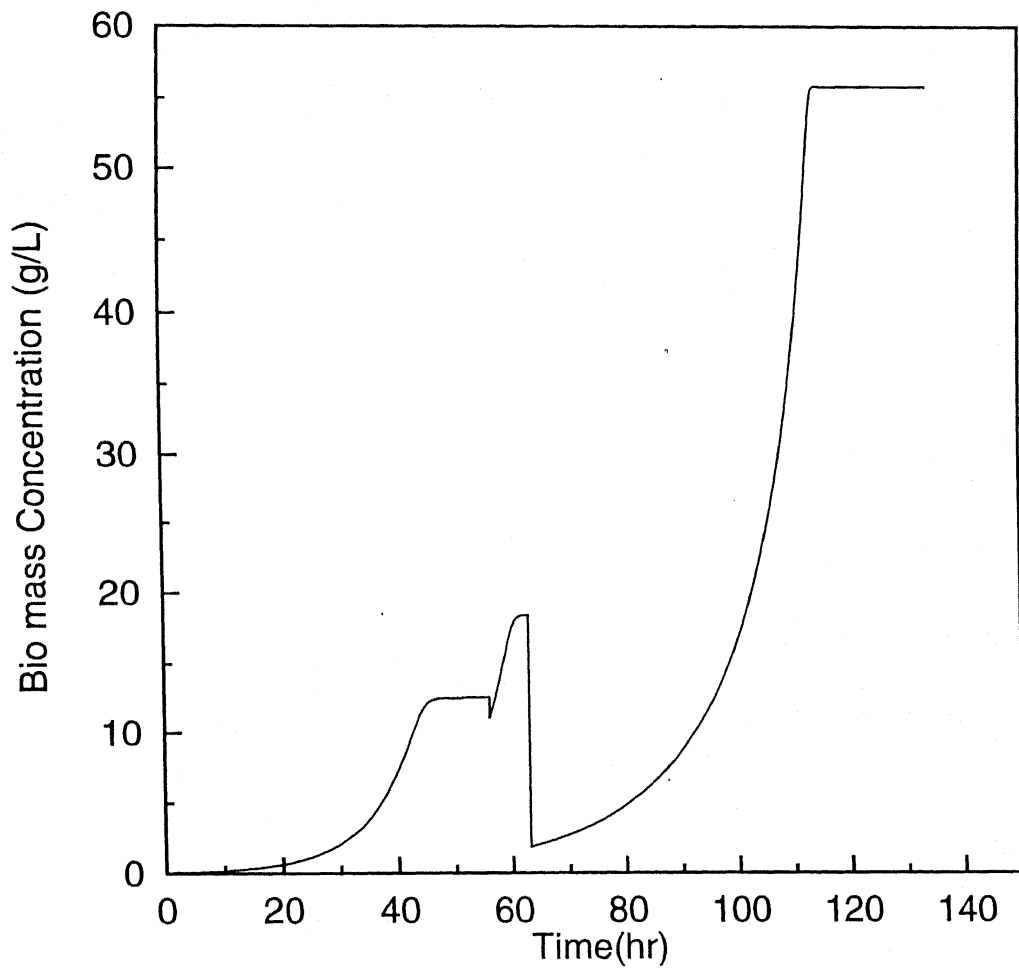


Fig. 4b(i) : Biomass concentration profile for the free  $V_f$  and  $t_f$  with unequal intervals.

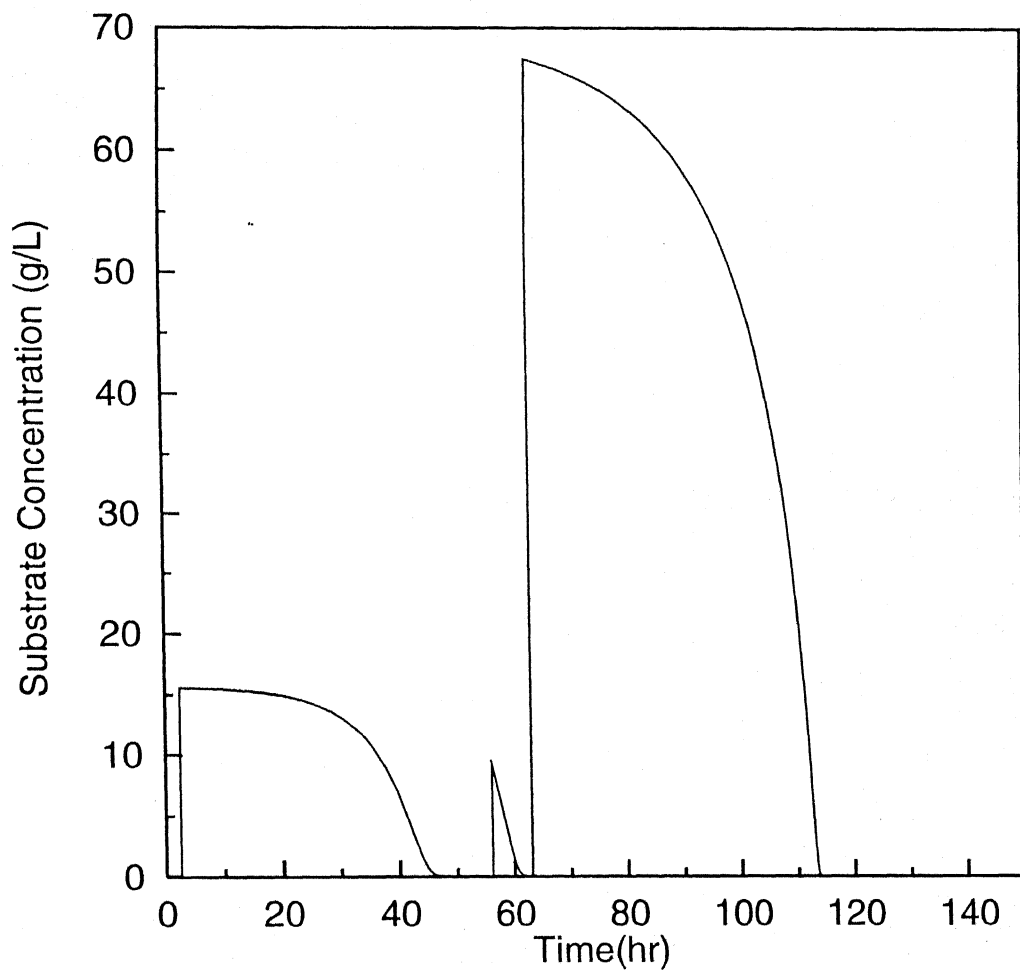


Fig. 4b(ii) : Substrate concentration profile for the free and  $t_f$  with unequal intervals.